such as DNA. Traditional magnetic tweezers geometry imposes a vertical extension on a surface-tethered molecule parallel to the microscope's optic axis, resulting in only a small section of DNA being in focus and thus being reliant on indirect measurements of magnetic bead position to infer topological dynamics. Here we describe a bespoke transverse magnetic tweezers system that can be integrated with optical tweezers and multicolor single-molecule fluorescence imaging. This permits independent control over the molecular extension and torque imposed on a single DNA molecule while allowing the full extent of the DNA to be visualized within the fluorescence microscope's depth-of-field, in addition to proteins bound to the DNA which affect its topology, such as DNA gyrase. Using a lamda DNA control we have generated a bifunctionalized construct via oligo insertion to permit tethering of single DNA molecules between a streptavidin-coated paramagnetic bead and a nanoscale platform, and have generated superresolution structural detail of DNA with video-rate sampling to a lateral precision of a few tens of nm utilizing the stochastic photoblinking of the intercalating DNA dye YOYO-1 and the minor groove binding dye SYTO-13 through Blinking assisted Localization Microscopy (BaLM).

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Tension-Dependent Stability of Torsionally Constrained DNA: Melting Precedes Overwinding

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Key genomic processes such as replication and transcription require localized unwinding and base-pair melting of the double-helix. *In vivo*, however, DNA is often torsionally constrained, presenting a topological barrier to unwinding. While it is known that unwinding can proceed through buckling of such DNA (resulting in plectonemes), this may only occur at low forces (typically below 1 pN). Here, we ask: how can torsionally constrained DNA unwind at higher forces without changing the overall twist?

Using a combination of optical tweezers and fluorescence microscopy we map the local changes in twist and base-pair integrity of torsionally constrained DNA as a function of tension and ionic strength. By correlating the second derivatives of force-extension curves of torsionally constrained DNA to the binding of a fluorescently-labeled single-stranded DNA binding protein (Replication Protein A), we identify two tension-dependent structural transitions. In agreement with a previous proposal, we show that at high tension (above 115 pN), unwinding is accompanied by the formation of overwound DNA (termed P-DNA) in a largely cooperative mechanism. Strikingly, we also reveal that for intermediate tensions (60 to 115 pN), localized base-pair melting can occur without changes in DNA twist. This mechanism, which is significantly less cooperative, is referred to here as 'pre-melting'. We demonstrate that pre-melting is favored by AT-rich sequences and low ionic strength. In contrast, P-DNA is largely stabilized by higher salt concentrations, while also having a preference for AT-rich domains. This supports the hypothesis that P-DNA consists of tightly entwined backbones with exposed bases.

These findings provide a new understanding of the interplay between DNA twist, extension and sequence. Moreover, since DNA unwinding can be induced through either tension or applied torque, this work may have strong implications for the metabolism of torsionally constrained DNA *in vivo*.

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Estimating Persistence Length of DNA from Molecular Dynamics Simulations

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Bending rigidity of DNA is one of its most important mechanical properties, since in eukaryotic cells a vast majority of the DNA (75-80%) is tightly packed into chromatin. Classical experiments have measured the persistence length (PL) of DNA, which is directly related to its bending rigidity, to be about 45-50 nm (140-160 bp). Previous all-atom molecular dynamics (MD) studies of DNA flexibility attempted to calculate this value using relatively small fragments (much less than PL) in explicit solvent. However, the simulations are usually computationally expensive due to a large number of water molecules required to construct the solvent box, and the resulting PL approximations are greater than the experimental values. One possible explanation for this discrepancy between experiment and simulation may be that classical experimental measurements were performed with DNA fragments on the order of PL or greater, while fragments much smaller than PL have so far been used in MD studies. In this work we provide a computationally inexpensive method of estimating the PL of DNA from atomistic MD simulations of long fragments on the order of PL (~150 bp) in implicit solvent that provides good agreement with experiment.

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Dual-Beam Fluorescence Correlation Spectroscopy of DNA Hairpin Folding-Unfolding Reaction at Various Temperatures and Salt Concentrations

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Fluorescence Correlation Spectroscopy has been used to study the conformational dynamics of DNA hairpin molecules of various loop lengths in aqueous solution. In a recent development, we are also able to monitor the temperature dependence of the processes of interest. In our 2-beam approach, the analyte molecules flow through an electrophoresis capillary under the influence of pressure driven flow or electrophoretic flow and are probed by two spatially offset probe volumes in such a way that the molecules flow sequentially from one probe volume to the next. A pair of dye-quencher molecules attached to DNA or RNA hairpin structures give rise to fluorescence fluctuations due to folding-unfolding dynamics of the hairpins. Fluorescence fluctuations from the probe volumes are analyzed using auto-correlation and cross-correlation analysis. In this way, we are able to resolve fluorescence fluctuation time constants arising from diffusion, flow, triplet blinking, and conformational fluctuations. Using a temperature controlling stage we can fix the temperature to within 0.2 degrees in the range 5 - 95 degrees Celsius, unaffected by the flow rate of the solution in the capillary. In this presentation, we will discuss recent results from our study of DNA hairpins of different loop lengths, at different temperatures and Sodium ion concentrations. We will also discuss binding and unbinding of counterions to individual nucleotides as they flow through the capillary under the influence of an applied electric field. Emphasis will be placed on how the desired information can be extracted using our unique approach to fluorescence correlation spectroscopy.

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Ion Binding and Electrostatics in Condensed DNA Arrays Probed by Ion Counting Techniques

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Despite decades of theoretical and experimental research, the mechanism of DNA condensation remains elusive. Numerous models successfully describe this condendsation, including those invoking dynamic counterion fluctuations, salt bridges, static counterion lattices, and models that invoke the underlying DNA geometry, all while presenting vastly different models for ion binding. Here we present new experimental data that quantifies the ions bound to condensed DNA arrays. By measuring the competition between condensing ions and various non-condensing ions we quantify the electrostatic interactions between the DNA and ions. Furthermore, by varying the inter-axial spacing of condensed DNA utilizing crowding agents, we report on the interplay between the packing force of DNA and competitive ion binding. Finally, we present a simple new Ion Binding Model (IBM) that captures much of the observed binding. These data should prove vital in determining the underlying electrostatic mechanisms that drive DNA condensation.

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Brownian Dynamics Study of DNA Supercoil Relaxation

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The stresses induced in DNA during cell processes such as replication and transcription lead to the formation of plectonemic supercoils. Supercoiling in turn affects these processes. As a result of this relationship between supercoiling and these processes, the degree of supercoiling in DNA needs to be controlled closely in order to optimize these cell processes. This control is facilitated by enzymes such as type 1b topoisomerases and nicking endonucleases which relax supercoiling in DNA.

The dynamics of DNA supercoil relaxation have been studied in recent experiments by means of single-molecule magnetic and/or optical tweezer experiments. Novel as these experiments are, they do not permit a direct observation of the structural changes that occur in DNA during supercoil relaxation. They depend on components (e.g. a paramagnetic bead) attached to the DNA to indirectly obtain information about these dynamics.

We studied the dynamics of supercoil relaxation by means of Brownian Dynamics simulations of a discrete wormlike-chain (dWLC) model of DNA. These simulations parallel the single-molecule experiments in which a single DNA molecule is held under constant tension so that its end-to-end extension increases as supercoils are relaxed by a nicking endonuclease. The dWLC model accounts for elasticity, electrostatics and entropic forces as well as for hydrodynamic interactions.